

Abstract No. mol210

Footprinting of the Interaction of the TATA Binding Protein (TBP) with DNA

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Beamline(s): X28C

Abstract: The "TATA Binding Protein (TBP) is a protein required for the initiation of transcription by each of the three eukaryotic RNA polymerases. The binding of TBP to specific promoter sequences ("TATA Boxes") is a key step in the initiation of transcription of genes transcribed by RNA polymerase II. The application of synchrotron x-ray nucleic acid and protein footprinting to protein-DNA interactions is being addressed through the binding of TBP to a number of naturally occurring promoters. TBP binding induces a dramatic and drastic conformational change in the structure of the bound DNA with two kinks in the DNA stabilized by the intercalation of two pairs of phenylalanine residues. Studies conducted during the past year focused upon resolving technical issues regarding protein stability, and in the stopped-flow mixing device, that had severely diminished the visibility of TBP footprints. These problems have been resolved through a new sample preparation protocol, the implementation of a new mixing T in the stopped-flow and an increase in the mixed sample volumes. We are now poised to restart the planned studies of TBP-TATA box interactions; preliminary studies are presently underway.